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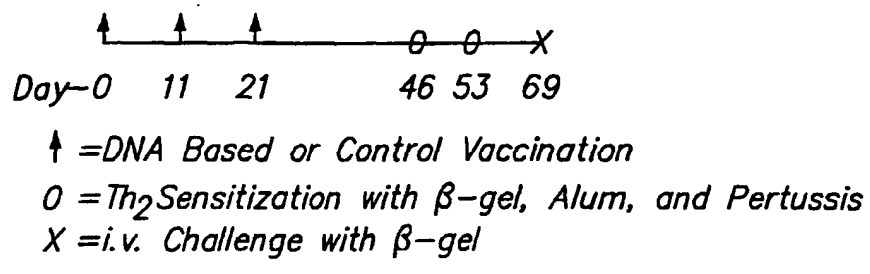
(54) Title: METHOD FOR PREVENTING AN ANAPHYLACTIC REACTION



(57) Abstract: The invention provides a method for reducing anaphylactic hypersensitivity response to an allergen in a subject. In one embodiment, the method comprises administering an immunomodulatory nucleic acid molecule to the subject. In another embodiment, the method comprises administering antigen with the immunomodulatory nucleic acid molecule to the subject, which antigen may be administered as a conjugate with or in admixure with the immnomodulatory nucleic acid molecule.

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METHOD FOR PREVENTING AN ANAPHYLACTIC REACTION

GOVERNMENT RIGHTS

This invention was made with Government support under Grant Nos. AI40682 and AI01490, awarded by the National Institutes of Health. The government may have certain rights in this invention.

FIELD OF THE INVENTION

The invention is in the field of immune response modulation, and in particular to reducing an anaphylactic hypersensitivity response.

BACKGROUND OF THE INVENTION

In humans, anaphylaxis is a life-threatening hypersensitivity response in which IgE/FcεRI mediated systemic mast cell degranulation occurs. However, only a small fraction of allergic individuals develop anaphylactic hypersensitivities. The true incidence of anaphylaxis and anaphylactic hypersensitivities is not known; however, one study proposes that 3.2 cases of anaphylaxis per 100,000 people occur each year, that 40% are misdiagnosed, and that 5% of cases are fatal. Sorenson et al. (1989) *Allergy* 44 :288-290. Of particular concern in the case of food allergies is that many anaphylactic reactions occur in individuals after the accidental injection of known allergens. Sampson et al. (1992) *N. Engl. J. Med.* 327:380-384.

Because of their lethal potential, effective therapies for the desensitization of individuals with anaphylactic hypersensitivities would be of great clinical utility. Unfortunately, except for stinging insects, traditional protein based immunotherapy (IT) has no established value in the desensitization of individuals at risk for anaphylaxis, and avoidance remains the only therapeutic option. Graft et al. (1993) Immunotherapy, p 934-944, in Bronchial Asthma Weiss and Stein, eds. 3rd ed. Little Brown & Co.; and Oppenheimer et al. (1992) *J. Allergy Clin. Immunol.* 90:256-262. In the case of protein immunotherapy, there is no single immunological parameter that reliably predicts successful anaphylactic desensitization in clinical practice. Ebner et al. (1999) *Allergy Immunol.* 119 :1-5 ; Gehlar et al. (1999) *Clin. Exp. Allergy* 29:497-506; and Golden et al. (1998) *J. Allergy Clin. Immunol.* 101-298-305. In the context of anaphylaxis, the antigen to which the individual is sensitive is considered an allergen. Anaphylactic hypersensitivity responses have even been demonstrated in individuals who have lost their skin test reactivity after receiving immunotherapy for five or more years. Golden et al. (1998) *J. Allergy Clin. Immunol.* 101-298-305. While DNA based immunization strategies have been shown to prevent the development of Th2 biased immune responses and to prevent

late phase allergic reactions in mouse and rat models of asthma, it has proven more difficult to prevent the early phase of the immediate hypersensitivity response, which characterizes anaphylaxis. Hsu et al. (1996) *Nature Med.* 2 :540-544 ; Slater et al. (1997) *J. Allergy Clin. Immunol.* 99:S504; Kohama et al. (1999) *J. Allergy Clin. Immunol.* 104:1231-1238; and Kline et al. (1998) *J. Immunol.* 160:2555-2559.

Traditional allergen immunotherapy has a limited role in allergen desensitization, mainly due to serious side effects. One of the side effects of this approach is an anaphylactic reaction that can lead to death. In the absence of any other effective therapeutic modality, allergen avoidance is generally the only safe option for preventing anaphylaxis.

There remains a need for treatment strategies that prevent the anaphylactic hypersensitivity response. The present invention addresses this need.

SUMMARY OF THE INVENTION

The invention provides methods for reducing the risk of an anaphylactic hypersensitivity response to an allergen in a subject. The invention further provides methods for reducing the severity of an anaphylactic hypersensitivity response to an antigen in a subject. In general, these methods comprise administering an immunomodulatory nucleic acid molecule to the subject. In some embodiments, the methods comprise co-administering antigen with the immunomodulatory nucleic acid molecule to the subject. In some of these embodiments, the antigen is administered in close spatial proximity to the immunomodulatory nucleic acid molecule. In particular embodiments, the antigen is an allergen, and the immunomodulatory nucleic acid molecule is administered as an allergen-immunomodulatory nucleic acid molecule conjugate. In other embodiments, the antigen is administered in admixture with the immunomodulatory nucleic acid molecule.

The invention provides an advantage over traditional, antigen-based desensitization therapies, in that untoward side effects, including death, associated with such therapies are avoided.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a timetable for vaccination, sensitization, and anaphylactic challenge.

Figure 2 is a graph depicting plasma histamine levels after anaphylactic challenge in pACB-LacZ vaccinated mice.

Figure 3 is a graph depicting serum antibody and cytokine responses of pACB-LacZ vaccinated mice.

Figure 4 is a graph depicting plasma histamine levels after anaphylactic challenge in βGal/ISS-ODN vaccinated mice.

Figure 5 is a graph depicting serum antibody and cytokine responses of β-Gal/ISS-ODN vaccinated mice.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the discovery that administration of immunomodulatory nucleic acid molecule to a subject reduces the risk of death due to anaphylactic hypersensitivity response. Anaphylactic allergens are those antigens that pose a risk of anaphylactic reaction in hypersensitive individuals.

Anaphylaxis is an acute, systemic allergic reaction that occurs after an individual has become sensitized to an antigen. Anaphylaxis is associated with the production of high levels of IgE antibodies and with the release of histamines, which cause muscle contractions, constriction of the airways, and dilation of blood vessels. Symptoms of anaphylactic reactions include hives, generalized itching, nasal congestion, wheezing, difficulty breathing, cough, cyanosis, lightheadedness, dizziness, confusion, slurred speech, rapid pulse, palpitations, nausea and vomiting, abdominal pain or cramping, skin redness or inflammation, nasal flaring, intercostals retractions, etc.

The invention provides a safer, and more potent therapeutic advantage, avoiding the disadvantages of traditional, antigen-based immunotherapy.

Before the present invention is described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

All scientific and technical terms used in this application have meanings commonly used in the art unless otherwise specified. As used in this application, the following words or phrases have the meanings specified.

It must be noted that as used herein and in the appended claims, the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an immunomodulatory nucleic acid molecule " includes a plurality of such molecules and reference to "the antigens" includes reference to one or more antigens and equivalents thereof known to those skilled in the art, and so forth.

Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to describe more fully the state of the art to which this invention pertains.

Definitions

As used herein, the terms "immunomodulatory nucleic acid molecule" refers to a polynucleotide that comprises at least one immunomodulatory nucleic acid moiety. Such immunomodulatory nucleic acid molecules are sometimes referred to in the art as "immunostimulatory sequences" or "ISS" or "ISS-ODN" because of previously described effects on an immune response.

The term "immunomodulatory," as used herein in reference to a nucleic acid molecule, refers to the ability of a nucleic acid molecule to modulate an immune response in a vertebrate host.

As used herein, "anaphylactic hypersensitivity response" refers to an immediate hypersensitivity reaction. Anaphylaxis can elicit systemic effects, or local (e.g., organ-specific) effects, which result in a change in vital signs. Anaphylactic hypersensitivity can lead to anaphylactic reaction or to anaphylactic shock.

The terms "oligonucleotide," "polynucleotide," and "nucleic acid molecule", used interchangeably herein, refer to a polymeric forms of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. The backbone of the polynucleotide can comprise sugars and phosphate groups (as may typically be found in RNA or DNA), or modified or substituted sugar or phosphate groups. Alternatively, the backbone of the polynucleotide can comprise a polymer of synthetic subunits such as phosphoramidites, and/or phosphorothioates, and thus can be an oligodeoxynucleoside phosphoramidate or a mixed phosphoramidate-phosphodiester oligomer. Peyrottes et al. (1996) *Nucl. Acids Res.* 24:1841-1848; Chaturvedi et al. (1996) *Nucl. Acids Res.* 24:2318-2323. The polynucleotide may comprise one or more L-nucleosides. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, uracyl, other sugars, and linking groups such as fluororibose and thioate, and nucleotide branches. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Other types of modifications included in this definition are caps, substitution of one or more of the naturally occurring nucleotides with an analog, and introduction of means for attaching the polynucleotide to proteins, metal ions, labeling components, other polynucleotides, or a solid support. Immunomodulatory nucleic acid molecules can be provided in various formulations, e.g., in association with liposomes, microencapsulated, etc., as described in more detail herein.

The terms "polypeptide," "peptide," and "protein", used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The term includes polypeptide chains modified or derivatized in any manner, including, but not limited to, glycosylation, formylation, cyclization, acetylation, phosphorylation, and the like. The term includes naturally-occurring peptides, synthetic peptides, and peptides comprising one or more amino acid analogs. The term includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and homologous

leader sequences, with or without N-terminal methionine residues; immunologically tagged proteins; and the like.

The terms "antigen" and "epitope" are well understood in the art and refer to the portion of a macromolecule which is specifically recognized by a component of the immune system, e.g., an antibody or a T-cell antigen receptor. As used herein, the term "antigen" encompasses antigenic epitopes, e.g., fragments of an antigen which are antigenic epitopes. Epitopes are recognized by antibodies in solution, e.g. free from other molecules. Epitopes are recognized by T-cell antigen receptor when the epitope is associated with a class I or class II major histocompatibility complex molecule. The term "antigen" includes allergenic antigens, or allergens. The term further includes allergens that have been modified, and extracts of allergens, e.g., crude extracts, which may comprise more than one allergen.

The terms "conjugated," "attached," and "linked" (and similar terms, e.g. "conjugation," "attachment," and "linkage") are used interchangeably herein to refer to a chemical association of two molecules, e.g., a nucleic acid molecule and a polypeptide. The chemical association may be covalent or non-covalent. The two molecules can be linked directly, or indirectly, e.g., via a linker ("spacer") molecule, a solid support, and the like.

As used herein, the terms "treatment", "treating", and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing (e.g., reducing the incidence of; reducing the risk of) a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse affect attributable to the disease and/or may be prophylactic in terms of completely or partially preventing (e.g., reducing the incidence of, reducing the risk of) a recurrence of the disease or symptom thereof. "Treatment", as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; (c) preventing recurrence of the disease in a subject which is predisposed to such a recurrence; and (d) relieving, or reducing the severity of, a symptom of the disease. Anaphylaxis is a life-threatening condition; therefore, "treatment," as used herein in the context of anaphylaxis, includes reducing the risk of death. In the context of an anaphylactic reaction, "treatment" refers to reducing the incidence of an anaphylactic reaction; reducing the risk that an anaphylactic reaction will occur in a susceptible individual; reducing an anaphylaxis-associated symptom or parameter; reducing the severity of an anaphylaxis-associated symptom or parameter; and reducing the severity of an anaphylactic reaction.

As used herein, "subject" or "individual" or "patient" refers to the recipient of the therapy to be practiced according to the invention. The subject is generally a mammal. If a mammal, the subject may be a human, but may also be a domestic livestock, laboratory subject or pet animal.

As used herein, "exposure" to a substance includes both natural, environmental exposure to the antigen as well as administration of the substance to a subject, and as such encompasses both accidental and deliberate exposure to the antigen.

THERAPEUTIC METHODS OF THE INVENTION

The present invention provides methods of reducing an anaphylactic reaction in an individual, methods of reducing a symptom of anaphylaxis, methods of reducing the risk of an anaphylactic response in an individual, and methods of reducing the incidence of anaphylaxis. The methods generally comprise administering an effective amount of an immunomodulatory nucleic acid molecule to an individual. The methods are useful to treat an anaphylactic response. Accordingly, the invention further provides methods of treating anaphylaxis in an individual.

The immunomodulatory nucleic acid molecule may be administered alone (e.g., without co-administration of antigen), in admixture with, or in spatial proximation with an antigen.

In some embodiments, the reduction in an anaphylactic response is antigen specific. The term "antigen-specific" is one well understood in the art, and as used herein refers to a reduction in an anaphylactic response to the antigen with which the individual is sensitized, or to closely related ("cross-reactive") antigens, e.g., antigens that share one or more epitopes with the sensitizing antigen.

Therapeutic methods of the invention reduce an anaphylaxis-associated parameter or symptom in an individual. As used herein, the term "an anaphylaxis-associated parameter or symptom" is a parameter or symptom associated with, or caused by, an anaphylactic reaction. Anaphylaxis-related symptoms and parameters include, but are not limited to, elevated levels of IgE antibodies, e.g., in the serum or other body fluid; release of histamines and other mediators produced by mast cells and/or basophils, including, but not limited to, leukotrienes (e.g., leukotriene A4), prostaglandins (e.g., prostaglandin D2), serotonin, eosinophil chemotactic factor, neutrophil chemotactic factor, proteases, platelet-activating factor, bradykinin, and cytokines (e.g., IL-1, TNF-α, IL-2, IL-3, IL-4, IL-5, IL-6, TGF-β, and GM-CSF); elevated serum levels of histamines; constriction of the airways; hives; generalized itching; nasal congestion; wheezing; difficult breathing; cough; cyanosis; lightheadedness; dizziness; confusion; slurred speech; rapid pulse; palpitations; nausea and vomiting; drop in blood pressure; edema; abdominal pain or cramping; skin redness or inflammation; nasal flaring; intercostals retraction; anaphylactic shock; and death.

An "effective amount" of an immunomodulatory nucleic acid molecule is one that reduces one or more anaphylaxis-associated symptom or parameter in an individual by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least

about 70%, at least about 80%, or at least about 90% or more, when compared with the symptom or parameter in the individual not treated with the immunomodulatory nucleic acid molecule, or when compared with a control individual not treated with the immunomodulatory nucleic acid molecule, e.g., a control individual exposed to an antigen but not treated with immunomodulatory nucleic acid molecule.

Whether a symptom or parameter associated with anaphylaxis has been reduced can be determined using any known assay or diagnostic method. Methods of measuring levels of histamine and other mediators produced by mast cells or basophils are well known in the art and can be used. In many instances, immunological assays are used, e.g., enzyme-linked immunosorbent assays, using antibody specific for a given mediator. Many such assays are commercially available. Methods of measuring histamine levels are described in the Examples.

Subjects suitable for treatment with the methods of the invention

Subjects particularly suitable for treatment with any of the subject methods include individuals known to be susceptible to having an anaphylactic reaction to a substance; and individuals suspected of being susceptible to having an anaphylactic reaction to a substance, when compared with a normal individual. For example, any patient with atopy, i.e., an individual who has had an allergic reaction, of any severity, to an antigen may be considered at risk of having an anaphylactic reaction to that antigen, and possibly to other antigens. As used herein, a "normal individual" is one who is not susceptible to having an anaphylactic reaction, i.e., under normal physiological conditions and under normal conditions of exposure to an allergen, a normal individual will not have an anaphylactic reaction or display symptoms of anaphylaxis.

Nucleic acid molecules comprising immunomodulatory nucleic acid molecule

Nucleic acid molecules comprising an immunomodulatory nucleic acid molecule which are suitable for use in the methods of the invention include an oligonucleotide, which can be a part of a larger nucleotide construct such as a plasmid. The term "polynucleotide" therefore includes oligonucleotides, modified oligonucleotides and oligonucleosides, alone or as part of a larger construct. The polynucleotide can be single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), single-stranded RNA (ssRNA) or double-stranded RNA (dsRNA).

As used herein the term "immunomodulatory nucleic acid molecule" encompasses an immunomodulatory nucleic acid molecule not conjugated with another moiety (e.g., an allergenic antigen); an immunomodulatory nucleic acid molecule in spatial proximation with an antigen; an immunomodulatory nucleic acid molecule conjugated with another moiety; an immunomodulatory nucleic acid molecule linked with another moiety; and an immunomodulatory nucleic acid molecule in admixture with one or more antigens. An immunomodulatory nucleic acid molecule may be administered alone or as part of a larger nucleic acid construct, as part of a conjugate with an antigen,

or otherwise in proximate association with an antigen. Thus, the term "immunomodulatory nucleic acid molecule," as used herein, encompasses an immunomodulatory nucleic acid molecule in proximate association with an antigen.

The polynucleotide portion can be linearly or circularly configured, or the oligonucleotide portion can contain both linear and circular segments. Modifications of oligonucleotides include, but are not limited to, modifications of the 3'OH or 5'OH group, modifications of the nucleotide base, modifications of the sugar component, and modifications of the phosphate group.

The immunomodulatory nucleic acid molecule can comprise ribonucleotides (containing ribose as the only or principal sugar component), deoxyribonucleotides (containing deoxyribose as the principal sugar component), or in accordance with the established state-of-the-art, modified sugars or sugar analogs may be incorporated in the oligonucleotide of the present invention. Examples of a sugar moiety that can be used include, in addition to ribose and deoxyribose, pentose, deoxypentose, hexose, deoxyhexose, glucose, arabinose, xylose, lyxose, and a sugar "analog" cyclopentyl group. The sugar may be in pyranosyl or in a furanosyl form. In the modified oligonucleotides of the present invention, the sugar moiety is preferably the furanoside of ribose, deoxyribose, arabinose or 2'-O-methylribose, and the sugar may be attached to the respective heterocyclic bases either in I or J anomeric configuration.

An immunomodulatory nucleic acid molecule may comprise at least one nucleoside comprising an L-sugar. The L-sugar may be deoxyribose, ribose, pentose, deoxypentose, hexose, deoxyhexose, glucose, galactose, arabinose, xylose, lyxose, or a sugar "analog" cyclopentyl group. The L-sugar may be in pyranosyl or furanosyl form.

The phosphorous derivative (or modified phosphate group) that can be attached to the sugar or sugar analog moiety in the modified oligonucleotides of the present invention can be a monophosphate, diphosphate, triphosphate, alkylphosphate, alkanephosphate, phosphoronthioate, phosphorodithioate or the like. The heterocyclic bases, or nucleic acid bases that are incorporated in the oligonucleotide base of the ISS can be the naturally occurring principal purine and pyrimidine bases, (namely uracil or thymine, cytosine, adenine and guanine, as mentioned above), as well as naturally occurring and synthetic modifications of said principal bases. Those skilled in the art will recognize that a large number of "synthetic" non-natural nucleosides comprising various heterocyclic bases and various sugar moieties (and sugar analogs) are available, and that the immunomodulatory nucleic acid molecule can include one or several heterocyclic bases other than the principal five base components of naturally occurring nucleic acids. Preferably, however, the heterocyclic base in the ISS is selected from uracil-5-yl, cytosin-5-yl, adenin-7-yl, adenin-8-yl, guanin-7-yl, guanin-8-yl, 4-aminopyrrolo [2,3-d] pyrimidin-5-yl, 2-amino-4-oxopyrolo [2,3-d] pyrimidin-5-yl, 2-amino-4-oxopyrrolo [2,3-d] pyrimidin-3-yl groups, where the purines are attached to the sugar moiety of the oligonucleotides via

the 9-position, the pyrimidines via the 1-position, the pyrrolopyrimidines via the 7-position and the pyrazolopyrimidines via the 1-position.

Structurally, the root oligonucleotide of the immunomodulatory nucleic acid molecule is a non-coding sequence that can include at least one unmethylated CpG motif. The relative position of any CpG sequence in ISS with immunomodulatory activity in certain mammalian species (e.g., rodents) is 5'-CG-3' (i.e., the C is in the 5' position with respect to the G in the 3' position).

Immunomodulatory nucleic acid molecules generally do not provide for, nor is there any requirement that they provide for, expression of any amino acid sequence encoded by the polynucleotide, and thus the sequence of a immunomodulatory nucleic acid molecule may be, and generally is, non-coding. Immunomodulatory nucleic acid molecules may comprise a linear double or single-stranded molecule, a circular molecule, or can comprise both linear and circular segments. Immunomodulatory nucleic acid molecules may be single-stranded, or may be completely or partially double-stranded.

In some embodiments, an immunomodulatory nucleic acid molecule is an oligonucleotide, e.g., consists of a sequence of from about 6 to about 200, from about 10 to about 100, from about 12 to about 50, or from about 15 to about 25, nucleotides in length.

Exemplary consensus CpG motifs of immunomodulatory nucleic acid molecules useful in the invention include, but are not necessarily limited to:

5'-Purine-Purine-[C]-[G]-Pyrimidine-Pyrimidine-3', in which the immunomodulatory nucleic acid molecule comprises a CpG motif flanked by at least two purine nucleotides (*e.g.*, GG, GA, AG, AA, II, *etc.*,) and at least two pyrimidine nucleotides (CC, TT, CT, TC, UU, *etc.*);

5'-Purine-TCG-Pyrimidine-Pyrimidine-3';

5'-$[TCG]_n$-3', where n is any integer that is 1 or greater, *e.g.*, to provide a poly-TCG immunomodulatory nucleic acid molecule (*e.g.*, where n=3, the polynucleotide comprises the sequence 5'-TCGTCGTCG-3'); and

5'-Purine-Purine -CG-Pyrimidine-Pyrimidine-CG-3'.

Exemplary DNA-based immunomodulatory nucleic acid molecules useful in the invention include, but are not necessarily limited to, polynucleotides comprising the following nucleotide sequences: AACGCC, AACGCT, AACGTC, AACGTT;
AGCGCC, AGCGCT, AGCGTC, AGCGTT;
GACGCC, GACGCT, GACGTC, GACGTT;
GGCGCC, GGCGCT, GGCGTC, GGCGTT;
ATCGCC, ATCGCT, ATCGTC, ATCGTT;
GTCGCC, GTCGCT, GTCGTC, GTCGTT; and

TCGTCG, and TCGTCGTCG.

Octameric sequences are generally the above-mentioned hexameric sequences, with an additional 3' CG. Exemplary DNA-based immunomodulatory nucleic acid molecules useful in the invention include, but are not necessarily limited to, polynucleotides comprising the following octameric nucleotide sequences:

AACGCCCG, AACGCTCG, AACGTCCG, AACGTTCG;
AGCGCCCG, AGCGCTCG, AGCGTCCG, AGCGTTCG;
GACGCCCG, GACGCTCG, GACGTCCG, GACGTTCG;
GGCGCCCG, GGCGCTCG, GGCGTCCG, GGCGTTCG;
ATCGCCCG, ATCGCTCG, ATCGTCCG, ATCGTTCG;
GTCGCCCG, GTCGCTCG, GTCGTCCG, and GTCGTTCG.

Immunomodulatory nucleic acid molecules useful in the invention can comprise one or more of any of the above CpG motifs. For example, immunomodulatory nucleic acid molecules useful in the invention can comprise a single instance or multiple instances (e.g., 2, 3, 5 or more) of the same CpG motif. Alternatively, the immunomodulatory nucleic acid molecules can comprises multiple CpG motifs (*e.g.*, 2, 3, 5 or more) where at least two of the multiple CpG motifs have different consensus sequences, or where all CpG motifs in the immunomodulatory nucleic acid molecules have different consensus sequences.

A non-limiting example of an immunomodulatory nucleic acid molecule has the sequence 5'-TGACTGTGAACGTTCGAGATGA-3' (SEQ ID NO:1). An example of a control nucleic acid molecule is one having the sequence 5'-TGACTGTGAAgGTTCGAGATGA-3' (SEQ ID NO:2), which differs from SEQ ID NO:1 at the nucleotide indicated in lower case type.

Immunomodulatory nucleic acid molecules useful in the invention may or may not include palindromic regions. If present, a palindrome may extend only to a CpG motif, if present, in the core hexamer or octamer sequence, or may encompass more of the hexamer or octamer sequence as well as flanking nucleotide sequences.

The core hexamer structure of the foregoing immunomodulatory nucleic acid molecules can be flanked upstream and/or downstream by any number or composition of nucleotides or nucleosides. However, ISS are at least 6 bases in length, and preferably are between 6 and 200 bases in length, to enhance uptake of the immunomodulatory nucleic acid molecule into target tissues.

In particular, immunomodulatory nucleic acid molecules useful in the invention include those that have hexameric nucleotide sequences having "CpG" motifs. Although DNA sequences are generally preferred, RNA immunomodulatory nucleic acid molecules can be used, with inosine and/or uracil substitutions for nucleotides in the hexamer sequences.

Modifications

Immunomodulatory nucleic acid molecules can be modified in a variety of ways. For example, the immunomodulatory nucleic acid molecules can comprise backbone phosphate group modifications (e.g., methylphosphonate, phosphorothioate, phosphoroamidate and phosphorodithioate internucleotide linkages), which modifications can, for example, enhance stability of the immunomodulatory nucleic acid molecule *in vivo,* making them particularly useful in therapeutic applications. A particularly useful phosphate group modification is the conversion to the phosphorothioate or phosphorodithioate forms of an immunomodulatory nucleic acid molecule. Phosphorothioates and phosphorodithioates are more resistant to degradation *in vivo* than their unmodified oligonucleotide counterparts, increasing the half-lives of the immunomodulatory nucleic acid molecules and making them more available to the subject being treated.

Other modified immunomodulatory nucleic acid molecules encompassed by the present invention include immunomodulatory nucleic acid molecules having modifications at the 5' end, the 3' end, or both the 5' and 3' ends. For example, the 5' and/or 3' end can be covalently or non-covalently conjugated to a molecule (either nucleic acid, non-nucleic acid, or both) to, for example, increase the bio-availability of the immunomodulatory nucleic acid molecules, increase the efficiency of uptake where desirable, facilitate delivery to cells of interest, and the like. Exemplary molecules for conjugation to the immunomodulatory nucleic acid molecules include, but are not necessarily limited to, cholesterol, phospholipids, fatty acids, sterols, oligosaccharides, polypeptides (*e.g.*, immunoglobulins), peptides, antigens (*e.g.*, peptides, small molecules, *etc.*), linear or circular nucleic acid molecules (*e.g.*, a plasmid), and the like. Additional immunomodulatory nucleic acid conjugates, and methods for making same, are known in the art and described in, for example, WO 98/16427 and WO 98/55495. Thus, the term "immunomodulatory nucleic acid molecule" includes conjugates comprising an immunomodulatory nucleic acid molecule.

Immunomodulatory nucleic acid molecules in association with antigen

An immunomodulatory nucleic acid molecule may be provided in an admixture (i.e., in solution) with antigen. An immunomodulatory nucleic acid molecule may be provided in a configuration such that it is proximately associated with antigen. In some embodiments, proximate association is achieved when an immunomodulatory nucleic acid molecule is conjugated, covalently or non-covalently, to an antigen, e.g., one or more allergens. In other embodiments, proximate association is achieved by means other than conjugation.

Admixtures

In some embodiments, an immunomodulatory nucleic acid molecule is in admixture with one or more antigens, e.g., one or more allergens, or an allergen extract. When an immunomodulatory nucleic acid molecule is in admixture with an antigen, the immunomodulatory nucleic acid molecule

and the antigen are present in solution at concentrations effective to reduce an anaphylactic reaction in an antigen-specific manner.

Spatial proximation

Spatial proximation (proximate association) can be accomplished in a number of ways, including, covalent or non-covalent association, linkage, or attachment, and can be by conjugation as well as means other than conjugation. Generally, an immunomodulatory nucleic acid molecule and an antigen are proximately associated at a distance sufficient or effective to reduce an anaphylactic reaction compared to administration of the immunomodulatory nucleic acid molecule and the antigen in admixture. Generally, the immunomodulatory nucleic acid molecule and the antigen are proximately associated at a distance of from about 0.1 angstroms to about 100 μm, from about 1 angstroms to about 10 μm, from about 10 angstroms to about 1 μm, from about 100 angstroms to about 100 nm, or from about 1 nm to about 10 nm, or any range intermediate or overlapping with the foregoing ranges.

Spatial proximation by conjugation

In some embodiments, an immunomodulatory nucleic acid molecule is conjugated, covalently or non-covalently, to an antigen. Thus, the term "immunomodulatory nucleic acid molecule" includes conjugates comprising an immunomodulatory nucleic acid molecule. Conjugates of nucleic acids an non-nucleic acid molecules, and methods for making same, are known in the art and described in, for example, WO 98/16427, WO 98/55495, WO 00/21556, each of which is incorporated by reference for their teachings relating to conjugates. Further teachings relating to nucleic acid conjugates may be found in S.L. Beaucage, ed. (1999) *Current Protocols in Nucleic Acid Chemistry*, John Wiley & Sons; and Kisakurek et al., eds. (2000) *Frontiers in Nucleic Acid Chemistry*, John Wiley & Sons.

Where the antigen is a peptide, the peptide portion of the conjugate can be attached to the immunomodulatory nucleic acid molecule through an amine, thiol, or carboxyl group in the peptide. If the peptide antigen contains a suitable reactive group (e.g., an N-hydroxysuccinimide ester) an immunomodulatory nucleic acid molecule can be reacted directly with an epsilon amino group of a lysine residue. The peptide portion of the conjugate can be attached to the 3' end of the immunomodulatory nucleic acid molecule through solid support chemistry. For example, the immunomodulatory nucleic acid molecule portion can be added to a polypeptide portion that has been pre-synthesized on a solid support (see, *e.g.*, Haralambidis et al. (1990) *Nucl. Acid. Res.* 18:493-499; Haralambidis et al. (1990) *Nucl. Acid. Res.* 18:501-505). Alternatively, the immunomodulatory nucleic acid molecule can be synthesized such that it is connected to a solid support through a cleavable linker extending from the 3' end. Upon chemical cleavage of the immunomodulatory nucleic acid molecule from the support, a terminal thiol group, or a terminal amino group, is left at the 3' end of the immunomodulatory nucleic acid molecule (*e.g.*, Zuckermann et al. (1987) *Nucl. Acids Res.* 15:5305-5321; Nelson et al. (1989) *Nucl. Acids. Res.* 17:1781-1794). Conjugation of an amino-

modified immunomodulatory nucleic acid molecule to amino groups of the peptide can be performed as described (see, *e.g.*, Benoit et al. (1987) *Neuromethods* 6:43-72). Conjugation of a thiol-modified immunomodulatory nucleic acid molecule to carboxyl groups of a peptide antigen can be performed as described (see, *e.g.*, Sinah et al. (1991) *Oligonucleotide Analogues: A Practical Approach*, IRL Press). Coupling of an immunomodulatory nucleic acid molecule carrying an appended maleimide to the thiol side chain of a cysteine residue of a peptide can also be performed (see, *e.g.*, Tung et al. (1991) *Bioconj. Chem.I* 2:464-465).

The peptide portion of a conjugate can be attached to the 5- end of an immunomodulatory nucleic acid molecule through an amine, thiol, or carboxyl group that has been incorporated into the immunomodulatory nucleic acid molecule during its synthesis (see, *e.g.*, Agrawal et al. (1986) *Nucleic Acids Res.* 14:6227-6245; Bischoff et al. (1987) *Anal. Biochem.* 164:336-344; and U.S. Patent Nos. 4,849,513; 5,015,733; 5,118,800; and 5,118,802).

The linkage of an immunomodulatory nucleic acid molecule to a lipid can be formed using standard known methods. These methods include, but are not limited to, the synthesis of oligonucleotide-phospholipid conjugates, oligonucleotide-fatty acid conjugates, and oligonucleotide-sterol conjugates (see, *e.g.*, Yanagawa et al. (1988) *Nucleic Acids Symp. Ser.* 19:189-192; Grabarek et al. (1990) *Anal. Biochem.* 185:131-135; and Boujrad et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:5728-5731).

Linkage of an immunomodulatory nucleic acid molecule to an oligosaccharide or polysaccharide can be performed using standard known methods, including, but not limited to, the method described in O'Shannessy et al. (1985) *J. Applied. Biochem.* 7:347-355.

An immunomodulatory nucleic acid molecule-antigen conjugate can be formed through covalent bonds, as described above. An immunomodulatory nucleic acid molecule-antigen conjugate can also be formed through non-covalent interactions, such as ionic bonds, hydrophobic interactions, hydrogen bonds, and/or van der Waals attractions.

Where the antigen is a polypeptide, the polypeptide may be proximately associated directly or indirectly to an immunomodulatory nucleic acid molecule, e.g., conjugated to the immunomodulatory nucleic acid molecule via a linker molecule. A wide variety of linker molecules are known in the art and can be used in the conjugates. The linkage from the peptide to the oligonucleotide may be through a peptide reactive side chain, or the N- or C-terminus of the peptide. Linkage from the oligonucleotide to the peptide may be at either the 3' or 5' terminus, or internal. A linker may be an organic, inorganic, or semi-organic molecule, and may be a polymer of an organic molecule, an inorganic molecule, or a co-polymer comprising both inorganic and organic molecules. A linker may also be a bead derivatized to contain appropriate groups for attachment of an immunomodulatory nucleic acid molecule and an

antigen. A wide variety of beads, including biodegradable beads, as well as methods of linking molecules to beads, are well known to those skilled in the art.

If present, the linker molecules are generally of sufficient length to permit oligonucleotides and/or polynucleotides and a linked polypeptide to allow some flexible movement between the oligonucleotide and the polypeptide. The linker molecules are generally about 6-50 atoms long. The linker molecules may also be, for example, aryl acetylene, ethylene glycol oligomers containing 2-10 monomer units, diamines, diacids, amino acids, or combinations thereof. Other linker molecules which can bind to oligonucleotides may be used in light of this disclosure.

Spatial proximation by means other than conjugation

In other embodiments, proximate association is achieved by means other than conjugation. An immumodulatory nucleic acid molecule can be proximately associated with one or more antigens in a number of ways. In some embodiments, an immumodulatory nucleic acid molecule and an antigen are proximately associated by encapsulation. In other embodiments, an immumodulatory nucleic acid molecule and an antigen are proximately associated by linkage to a platform molecule. A "platform molecule" is a molecule containing sites which allow for attachment of an immumodulatory nucleic acid molecule and one or more antigens. In other embodiments, an immumodulatory nucleic acid molecule and an antigen are proximately associated by adsorption onto a surface, such as a carrier particle.

In embodiments in which an immumodulatory nucleic acid molecule and an antigen are proximately associated by linkage to a platform molecule (also referred to herein as a "platform"), the platform may be proteinaceous or non-proteinaceous. A platform is generally multivalent (i.e., contains more than one binding, or linking, site) to accommodate binding to both an immumodulatory nucleic acid molecule and an antigen. Examples of proteinaceous platforms include, but are not limited to, albumin, gammaglobulin, immunoglobulin, and ovalbumin. Borel et al. (1990) *Immunol. Methods* 126:159-168; Dumas et al. (1995) *Arch. Dermatol. Res.* 287:123-128; Borel et al. (1996) *Ann. N.Y. Acad. Sci.* 778:80-87. Other examples of polymeric platforms are dextran, polyacrylamide, ficoll, carboxymethylcellulose, polyvinyl alcohol, polyvinylpyrrolidone, and poly D-glutamic acid/D-lysine. Other examples of platforms suitable for use within the present invention are chemically-defined, non-polymeric valency platforms such as those disclosed in U.S. Patent No. 5,552,391. Further examples of suitable platforms are derivatized 2,2'-ethylenedioxydiethylamine (EDDA); triethylene glycol (TEG); tetraaminobenzene; heptaaminobetacyclodextrin; tatraaminopentaerythritol; 1,4,8,11-tetraazacyclotetradecane; and 1,4,7,10-tetraazacyclododecane.

Conjugation of an immumodulatory nucleic acid molecule and an antigen to a platform may be effected in any of a number of ways, generally involving one or more crosslinking agents and

functional groups on the antigen, the immumodulatory nucleic acid molecule, and the platform molecule. Linking groups are added to platforms using standard synthetic chemistry techniques.

The principles of using platform molecules are well understood in the art. Generally, a platform contains, or is derivatized to contain, appropriate binding sites for immumodulatory nucleic acid molecules and antigen molecules. In addition, or alternatively, the immumodulatory nucleic acid molecule and/or the antigen is derivatized to provide appropriate linkage groups.

Where the antigen and immunomodulatory nucleic acid molecule are proximately associated by encapsulation, suitable encapsulating agents include, but are not limited to, oil-in-water emulsions, microspheres, beads, macromoleculare complexes, nanocapsules, micelles, mixed micelles, microparticles, and liposomes. The encapsulation composition may further comprise any of a wide variety of components, including, but not limited to, alum, lipids, surfactants, targeting moieties, phospholipids, lipid membrane structures, polyethylene glycol, and other polymers, such as polypeptides, glycopeptides, and polysaccharides. Encapsulating agents, as well as methods of making same, have been amply described in the literature, and need not be elaborated upon herein. For example, WO 00/21556, which provides a discussion of various encapsulating agents, is incorporated by reference herein for its teachings relating to encapsulating agents and methods of encapsulating a nucleic acid molecule and an antigen.

In embodiments in which an immumodulatory nucleic acid molecule and an antigen are proximately associated by adsorption onto a surface, the surface may be in the form of a carrier particle made with either an inorganic or organic core. Suitable carrier particles include, but are not limited to, nanocrystalline particles, activated carbon particles, and protein-ceramic nanoplates. Adsorption of nucleic acid molecules and other molecules such as polypeptides is well known in the art. See, for example, Douglas et al. (1987) *Crit. Rev. Ther. Drug Carrier Syst.* 3:233-261; Bousquet et al. (1999) *Pharm. Res.* 16:141-147; and U.S. Patent No. 5,460,831. The adsorbent surface may be biodegradable. Adsorption of an immumodulatory nucleic acid molecule and/or an antigen to a surface may occur through non-covalent interactions.

Antigens

Of interest in the methods of the invention are immunomodulatory nucleic acid molecules in admixture, or in spatial proximation, with an antigen, which may be an allergen. Antigens encompass a broad class of compounds, including, but not limited to, polypeptides, polysaccharides, glycoproteins, lipoproteins, and lipopolysaccharides. Methods for preparing antigens of all types are well known in the art and need not be elaborated upon herein.

Allergens

Allergens are immunogenic compounds that cause Th2-type T cell responses and IgE B cell responses, mast cell and basophil degranulation, and release of histamine and other mediators in

susceptible individuals. As used herein, "allergen" encompasses purified allergen, allergenic fragments of an allergen, extracts from a source of allergen, and the like. Allergens of interest include food allergens, chemical allergens (e.g., drugs, cosmetics, and the like), plant-derived allergens, and animal-derived allergens. Allergens of interest according to the present invention include antigens found in foods such as fruits (e.g., melons, strawberries, pineapple and other tropical fruits), peanuts, peanut oil, other nuts, milk proteins, egg whites, shellfish, tomatoes, etc.; airborne antigens such as grass pollens, animal danders, house mite feces, etc.; drug antigens such as penicillins and related antibiotics, sulfa drugs, barbiturates, anticonvulsants, insulin preparations (particularly from animal sources of insulin), local anesthetics (e.g., Novocain), and iodine (found in many X-ray contrast dyes); insect venoms and agents responsible for allergic dermatitis caused by blood sucking arthropods such as Diptera, including mosquitos (Anopheles sp., Aedes sp., Culiseta sp., Culex sp.), flies (Phlebotomus sp., Culicoides sp.) particularly black flies, deer flies and biting midges, ticks (Dermmacenter sp., Omithodoros sp., Otobius sp.), fleas (e.g., the order Siphonaptera, including the genera Xenopsylla, Pulex and Ctenocephalides felis felis); and latex. The specific allergen may be any type of chemical compound such as, for example, a polysaccharide, a fatty acid moiety, a protein, etc. Antigen preparations may be prepared by any available technique including, for example, isolation from natural sources, in vivo or in vitro expression of recombinant DNA molecules (see, for example, Zeiler et al. (1997) J. Allergy Clin. Immunol. 100(6 Pt 1):721-727, chemical synthesis, or other technique known in the art.

A wide variety of allergen preparations are available in the art, and many allergens have been molecularly cloned. For example, cloned allergens include Dermatophagoides pteryonyssinus (Der P1); Lol pl-V from rye grass pollen; various insect venoms including venom from jumper ant Myrmecia pilosula, Apis mellifera bee venom phospholipase A2 (PLA.sub.2) and antigen 5S, phospholipases from the yellow jacket Vespula maculifrons and white faced hornet Dolichovespula maculata; a large number of pollen proteins including birch pollen, ragweed pollen, Parol (the major allergen of Parietaria oficinalis) and the cross-reactive allergen Parjl (from Parietaria judaica) and other atmospheric pollens including Olea europaea, Artemisia sp., gramineae, etc.

Polypeptide antigens

A polypeptide antigen may be conjugated directly or indirectly, e.g., via a linker molecule, to an immunomodulatory nucleic acid molecule. A wide variety of linker molecules are known in the art and can be used in the conjugates. The linkage from the peptide to the oligonucleotide may be through a peptide reactive side chain, or the N- or C-terminus of the peptide. Linkage from the oligonucleotide to the peptide may be at either the 3' or 5' terminus, or internal. A linker may be an organic, inorganic, or semi-organic molecule, and may be a polymer of an organic molecule, an inorganic molecule, or a co-polymer comprising both inorganic and organic molecules.

If present, the linker molecules are generally of sufficient length to permit oligonucleotides and/or polynucleotides and a linked polypeptide to allow some flexible movement between the oligonucleotide and the polypeptide. The linker molecules are generally about 6-50 atoms long. The linker molecules may also be, for example, aryl acetylene, ethylene glycol oligomers containing 2-10 monomer units, diamines, diacids, amino acids, or combinations thereof. Other linker molecules which can bind to oligonucleotides may be used in light of this disclosure.

Peptides may be synthesized chemically or enzymatically, may be produced recombinantly, may be isolated from a natural source, or a combination of the foregoing. Peptides may be isolated from natural sources using standard methods of protein purification known in the art, including, but not limited to, HPLC, exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique. One may employ solid phase peptide synthesis techniques, where such techniques are known to those of skill in the art. *See* Jones, The Chemical Synthesis of Peptides (Clarendon Press, Oxford)(1994). Generally, in such methods a peptide is produced through the sequential additional of activated monomeric units to a solid phase bound growing peptide chain. Well-established recombinant DNA techniques can be employed for production of peptides.

Preparation of immunomodulatory nucleic acid molecules

Immunomodulatory nucleic acid moleculse can be synthesized using techniques and nucleic acid synthesis equipment that are well known in the art. For reference in this regard, see, e.g., Ausubel, et al., Current Protocols in Molecular Biology, Chs. 2 and 4 (Wiley Interscience, 1989); Maniatis, et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Lab. New York, 1982); U.S. Patent No. 4,458,066 and U.S. Patent No. 4,650,675. Because the immunomodulatory nucleic acid molecule is non-coding, there is no concern about maintaining an open reading frame during synthesis.

Alternatively, an immunomodulatory nucleic acid molecule can be isolated from microbial species using techniques well known in the art, such as nucleic acid hybridization. Whole are fragmented bacterial DNA can be used. Preferably, such isolated immunomodulatory nucleic acid molecules will be purified to a substantially pure state; i.e., to be free of endogenous contaminants, such as lipopolysaccharides.

Formulations

In general, immunomodulatory nucleic acid molecules are prepared in a pharmaceutically acceptable composition for delivery to a host. Pharmaceutically acceptable carriers preferred for use with the immunomodulatory nucleic acid molecules in the subject methods may include sterile aqueous of non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/ aqueous solutions, emulsions or suspensions, and microparticles, including saline and buffered media. Parenteral vehicles include

sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. A composition comprising a immunomodulatory nucleic acid molecule may also be lyophilized using means well known in the art, for subsequent reconstitution and use according to the invention. Also of interest are formulations for liposomal delivery, and formulations comprising microencapsulated immunomodulatory nucleic acid molecules.

In general, the pharmaceutical compositions can be prepared in various forms, such as granules, tablets, pills, suppositories, capsules, suspensions, salves, lotions and the like. Pharmaceutical grade organic or inorganic carriers and/or diluents suitable for oral and topical use can be used to make up compositions comprising the therapeutically active compounds. Diluents known to the art include aqueous media, vegetable and animal oils and fats. Stabilizing agents, wetting and emulsifying agents, salts for varying the osmotic pressure or buffers for securing an adequate pH value, and skin penetration enhancers can be used as auxiliary agents. Preservatives and other additives may also be present such as, for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like.

Immunomodulatory nucleic acid molecules can be administered in the absence of agents or compounds that might facilitate uptake by target cells (*e.g.*, as a "naked" polynucleotide, *e.g.*, a polynucleotide that is not encapsulated by a viral particle). Immunomodulatory nucleic acid molecules can be administered with compounds that facilitate uptake of immunomodulatory nucleic acid molecules by target cells (*e.g.*, by macrophages) or otherwise enhance transport of an immunomodulatory nucleic acid molecule to a treatment site for action. Absorption promoters, detergents and chemical irritants (e.g., keratinolytic agents) can enhance transmission of an immunomodulatory nucleic acid molecule composition into a target tissue (*e.g.*, through the skin). For general principles regarding absorption promoters and detergents which have been used with success in mucosal delivery of organic and peptide-based drugs, see, *e.g.*, Chien, *Novel Drug Delivery Systems*, Ch. 4 (Marcel Dekker, 1992). Examples of suitable nasal absorption promoters in particular are set forth at Chien, *supra* at Ch. 5, Tables 2 and 3; milder agents are preferred. Suitable agents for use in the method of this invention for mucosal/nasal delivery are also described in Chang, *et al.*, *Nasal Drug Delivery*, "Treatise on Controlled Drug Delivery", Ch. 9 and Tables 3-4B thereof, (Marcel Dekker, 1992). Suitable agents which are known to enhance absorption of drugs through skin are described in Sloan, Use of Solubility Parameters from Regular Solution Theory to Describe Partitioning-Driven Processes, Ch. 5, "Prodrugs: Topical and Ocular Drug Delivery" (Marcel Dekker, 1992), and at places elsewhere in the text. All of these references are incorporated herein for the sole purpose of illustrating the level of knowledge and skill in the art concerning drug delivery techniques.

A colloidal dispersion system may be used for targeted delivery of the immunomodulatory nucleic acid molecules to specific tissue. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes.

Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 Fm can encapsulate a substantial percentage of an aqueous buffer comprising large macromolecules. RNA and DNA can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, *et al.*, (1981) *Trends Biochem. Sci.*, 6:77). The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations. Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

Where desired, targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various well known linking groups can be used for joining the lipid chains to the targeting ligand (see, e.g., Yanagawa, *et al.*, (1988) *Nuc. Acids Symp. Ser.*, 19:189; Grabarek, *et al.*, (1990) *Anal. Biochem.*, 185:131; Staros, *et al.*, (1986) *Anal. Biochem.* 156:220 and Boujrad, *et al.*, (1993) *Proc. Natl. Acad. Sci. USA*, 90:5728). Targeted delivery of immunomodulatory nucleic acid molecules can also be achieved by conjugation of the immunomodulatory nucleic acid molecules to a the surface of

viral and non-viral recombinant expression vectors, to an antigen or other ligand, to a monoclonal antibody or to any molecule which has the desired binding specificity.

Routes of administration

Immunomodulatory nucleic acid molecules are administered to an individual using any available method and route suitable for drug delivery, including *in vivo* and *ex vivo* methods, as well as systemic, mucosal, and localized routes of administration.

Conventional and pharmaceutically acceptable routes of administration include intranasal, intramuscular, intratracheal, intratumoral, subcutaneous, intradermal, topical application, intravenous, rectal, nasal, oral and other parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the immunomodulatory nucleic acid molecule and/or the desired effect on the immune response. The immunomodulatory nucleic acid composition can be administered in a single dose or in multiple doses, and may encompass administration of booster doses, to elicit and/or maintain the desired effect on the immune response.

Immunomodulatory nucleic acid molecules can be administered to a host using any available conventional methods and routes suitable for delivery of conventional drugs, including systemic or localized routes. In general, routes of administration contemplated by the invention include, but are not necessarily limited to, enteral, parenteral, mucosal (e.g., intranasal), or inhalational routes. Inhalational routes may be preferred in cases of pulmonary involvement, particularly in view of the activity of certain immunomodulatory nucleic acid molecules in reducing an anaphylactic response.

Inhalational routes of administration (*e.g.*, intranasal, intrapulmonary, and the like) are of interest. Such means include inhalation of aerosol suspensions or insufflation of the polynucleotide compositions described herein. Nebulizer devices, metered dose inhalers, and the like suitable for delivery of polynucleotide compositions to the nasal mucosa, trachea and bronchioli are well known in the art and will therefore not be described in detail here. For general review in regard to intranasal drug delivery, see, *e.g.*, Chien, *Novel Drug Delivery Systems,* Ch. 5 (Marcel Dekker, 1992).

Parenteral routes of administration other than inhalation administration include, but are not necessarily limited to, topical, ophthalmic, transdermal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, intranasal, intraocular, and intravenous routes, *i.e.*, any route of administration other than through the alimentary canal. Parenteral administration can be carried to effect systemic or local delivery of immunomodulatory nucleic acid molecules. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations. Immunomodulatory nucleic acid molecules can also be delivered to the subject by enteral administration. Enteral routes of administration include, but are not necessarily limited to, oral and rectal (*e.g.*, using a suppository) delivery.

Methods of administration of immunomodulatory nucleic acid molecules through the skin or mucosa include, but are not necessarily limited to, topical application of a suitable pharmaceutical preparation, transdermal transmission, injection and epidermal administration. For transdermal transmission, absorption promoters or iontophoresis are suitable methods. For review regarding such methods, those of ordinary skill in the art may wish to consult Chien, *supra* at Ch. 7. Iontophoretic transmission may be accomplished using commercially available "patches" which deliver their product continuously via electric pulses through unbroken skin for periods of several days or more. An exemplary patch product for use in this method is the LECTRO PATCH™ (manufactured by General Medical Company, Los Angeles, CA) which electronically maintains reservoir electrodes at neutral pH and can be adapted to provide dosages of differing concentrations, to dose continuously and/or to dose periodically.

Epidermal administration can be accomplished by mechanically or chemically irritating the outermost layer of the epidermis sufficiently to provoke an immune response to the irritant. An exemplary device for use in epidermal administration employs a multiplicity of very narrow diameter, short tynes which can be used to scratch immunomodulatory nucleic acid molecules coated onto the tynes into the skin. The device included in the MONO-VACC™ tuberculin test (manufactured by Pasteur Merieux, Lyon, France) is suitable for use in epidermal administration of immunomodulatory nucleic acid molecules.

The invention also contemplates opthalmic administration of immunomodulatory nucleic acid molecules, which generally involves invasive or topical application of a pharmaceutical preparation to the eye. Eye drops, topical creams and injectable liquids are all examples of suitable formulations for delivering drugs to the eye.

In some embodiments, immunomodulatory nucleic acid molecule and antigen are co-administered. In these embodiments, the immunomodulatory nucleic acid molecule and the antigen may be administered substantially simultaneously, or the immunomodulatory nucleic acid molecule may be administered before or after the antigen. When co-administered, the immunomodulatory nucleic acid molecule and the antigen are generally administered within about 72 hours, about 48 hours, about 24 hours, about 12 hours, about 8 hours, about 4 hours, about 2 hours, about 1 hour, or about 30 minutes or less, of each other. Antigen may be administered separately from the immunomodulatory nucleic acid molecule, in admixture with immunomodulatory nucleic acid molecule, or proximately associated with (e.g., conjugated or brought into spatial proximation by other means, as described in more detail below) to one or more immunomodulatory nucleic acid molecules.

Dosages

Although the dosage used will vary depending on the clinical goals to be achieved, a suitable dosage range is one that provides up to about 1 μg to about 1,000 μg or about 10,000 μg of

immunomodulatory nucleic acid molecule can be administered in a single dosage. Alternatively, a target dosage of immunomodulatory nucleic acid molecule can be considered to be about 1-10 μM in a sample of host blood drawn within the first 24-48 hours after administration of immunomodulatory nucleic acid molecules. In general, doses of allergen are those which are in practice for allergen-based immunotherapy. Based on current studies, immunomodulatory nucleic acid molecules are believed to have little or no toxicity at these dosage levels.

It should be noted that the immunotherapeutic activity of immunomodulatory nucleic acid molecules is generally dose-dependent. Therefore, to increase immunomodulatory nucleic acid molecules potency by a magnitude of two, each single dose is doubled in concentration. Increased dosages may be needed to achieve the desired therapeutic goal. The invention thus contemplates administration of multiple doses to provide and maintain a reduction in a symptom associated with anaphylaxis. For example, immunomodulatory nucleic acid molecules may be administered at intervals ranging from at least every two weeks to every four weeks (*e.g.*, monthly intervals) (*e.g.*, every four weeks).

In view of the teaching provided by this disclosure, those of ordinary skill in the clinical arts will be familiar with, or can readily ascertain, suitable parameters for administration of immunomodulatory nucleic acid molecule according to the invention, including combination of immunomodulatory nucleic acid molecule administration with conventional treatments to prevent anaphylactic reaction such as anti-histamines, glucosteroids, and the like.

EXAMPLES

Example 1: Protection against the development of anaphylactic hypersensitivity

Materials and Methods

Mice: Female 6-8 wk C3H/HeJ mice were purchased from Jackson Laboratory (Bar Harbor, ME). All mice were maintained in the University of California, San Diego Animal Facility, which is certified by the American Association for the Accreditation of Laboratory Animal Care, and all experiments were approved by the animal care committee at our institution.

Reagents: pACB-LacZ contains the CMV IE1 promoter-intron , the simian virus 40 t-intron, the E. coli LacZ cDNA, and the simian virus 40 polyadenylation site. pACB is the same construct without the E. coli LacZ insert. Plasmids were purified using a megaprep kit (Qiagen, Chatsworth, CA), and endotoxin was removed by extraction with Triton X-114 (Sigma, St Louis, MO) to below the limits of detection (0.005 EU/ml) by limulus amoebocyte lysate assay (Bio-Whittaker, Walkersville, MD). β-galactosidase (β-gal), and pertussis toxin were obtained from Sigma (St Louis, MO). Phosphorothioate ISS-ODN and mutated oligodeoxynucleotide were purchased from Trilink Biotechnologies (San Diego, CA). The ISS-ODN used in these studies has the sequence 5'-TGACTGTGAACGTTCGAGATGA-3'

(SEQ ID NO:1) and the mutated oligodeoxynucleotide (M-ODN) has the sequence 5'-TGACTGTGAACCTTCCAGATGA-3' (SEQ ID NO:2). Endotoxin could not be detected in these oligodeoxynucleotide preparations by limulus amoebocyte lysate assay (Bio-Whittaker).

Vaccination, sensitization, and anaphylactic challenge protocols: The timetable for vaccination, sensitization, and anaphylactic challenge is shown in Figure 1. Mice received intradermal (i.d.) injections with 50μg of pACB-LacZ or pACB, or β-gal (10μg) alone, or mixed with ISS-ODN (10μg) or M-ODN (10μg), in 50μl normal saline, in the base of the tail, on 3 occasions, ten days apart. Twenty five days after the last vaccination, the mice were Th_2 sensitized with a mixture of β-gal (100μg), pertussis toxin (300ng), and alum (1mg), in 500μl normal saline injected intraperitoneally (i.p.) on 2 occasions 7 days apart. Mice received an intravenous (i.v.) challenge with 150μg of β-gal in 50μl normal saline 3 weeks after their last i.p. sensitization. The mice were then observed for one hour after i.v. anaphylactic challenge because preliminary experiments demonstrated that challenged mice either died or recovered from challenge in this first hour. Ten minutes before, and 2 minutes after challenge a subset of mice were bled and plasma collected in EDTA tubes for histamine analysis using a commercial ELISA kit according to the manufacturers recommendations (IMMUNOTECH, Westbrook, MN). Mice used to assess histamine release were not used in the final analysis of death from anaphylactic challenge.

Antibody assays: Serum β-gal specific IgG2a and IgG1 were measured by ELISA as previously described (13, 18,19,21). Results are expressed in units/ml (U/ml) based on pooled high titer anti-β-gal IgG2a and IgG1 standards which were given arbitrary concentrations of 4,000 U/ml. Ninety six well plates were coated with 5μg/ml of β-gal (Sigma) in 50 μl BBS (pH-9.2) overnight at 4°C. Plates were then blocked with 1%BSA in BBS at 37°C for 2 hours, washed with BBS/0.5% Tween 20 (Sigma), and incubated with standards and samples overnight at 4°C. Plates were then incubated with alkaline phosphatase linked anti-IgG1 or IgG2a (Southern Biotechnologies, Birmingham, AL) at a 1:2000 dilution, washed, and then incubated with p-nitrophenyl phosphate (2.63 mg/ml; Boehringer Mannheim). Absorbance at 405nm-650nm was read at 1 hour. Sample concentrations were calculated by comparison with the standard curve on each plate using the DeltaSOFT II v. 3.66 program (Biometallics, Princeton, NJ).

Antigen specific serum IgE titers were also measured by ELISA, and results are expressed in U/ml based on a pooled high titer anti-β-gal IgE standard given an arbitrary value of 2,560 U/ml. In order to remove antigen specific IgG, serum samples were incubated with protein G sepharose beads according to the manufacturer's recommendations (Pharmacia, Piscataway, NJ). Ninety six well plates were coated withβ-gal at 5μg/ml in 0.05M carbonate buffer and non-specific binding sites were saturated by incubation of plates with 1% BSA in BBS. Protein G absorbed 1:10 and 1:40 dilutions of sera were added and the plates incubated overnight at 4°C. Plates were then washed and incubated with goat

biotinylated anti-mouse IgE at 8μg/ml (Pharmingen, San Diego, CA). Plates were subsequently washed and incubated with horseradish peroxidase linked strepavidin at a 1:2000 dilution (Zymed, San Francisco, CA) and then TMB substrate (3,3',5,5'-tetramethyl benzidine, Kirkegaard and Perry Laboratories, Gaithersburg, MD). The color reaction was stopped with an equal volume of 1M phosphoric acid. Absorbance at 450nm-650nm was read and compared to the standard curve on each plate using the DeltaSOFT II v. 3.66 program (Biometallics, Princeton, NJ).

Splenocyte cytokine profiles: Antigen specific splenocyte cytokine profiles were assessed as previously described. Sato et al. (1996) *Science* 273 :352-354 ; Roman et al. (1997) *Nature Med.* 3:849-854; and Horner et al. (1998) *Cell. Immunol.* 190:77-82. Briefly, mouse spleens were harvested after i.v. challenge, teased to prepare single cell suspensions and resuspended in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 2mM-glutamine, β2-mercaptoethanol, and 1% penicillin-streptomycin (complete media). Splenocytes were incubated at 5 X 10^5 cells per well in 96 well plates in a final volume of 200μl of complete media with β-gal added at 10μg/ml at 37°C/5% CO_2. Culture supernatants were harvested at 72 hours and analyzed by ELISA. Samples were tested for the presence of IL-4, IL-5, and IFNγ by ELISA using capture and biotinylated detecting antibodies for IL-4 (Genzyme, San Francisco), IL-5 (Pharmingen) and IFNγ (Pharmingen). Washing and blocking steps were analogous to those used in the Ig ELISA described previously. Detection of the biotinylated secondary antibody was performed by adding 1:2000 diluted horseradish peroxidase (HP)-labeled streptavidin (Zymed) followed by TMB peroxidase substrate reagent (Kirkegaard and Perry Laboratories Incorporated). The color reaction was stopped with an equal volume of 1M phosphoric acid and absorbance was read at 450nm-650nm. A standard curve was generated using known amounts of recombinant IL-4, IL-5 and IFNγ (Genzyme and Pharmingen). Each supernatant was compared to the standard curve on the plate to quantitate cytokine levels using the DeltaSOFT II v. 3.66 program.

Statistics: Statistical analysis was conducted using Statview and Mathsoft computer software. Death as an outcome variable was compared between groups using the Fisher's exact test. Two tailed unpaired student t tests were conducted to compare histamine, antibody, and cytokine histamine levels.

Results

pDNA vaccination provides protection against the development of anaphylactic hypersensitivity: In preliminary studies, 6-8 week old female C3H/HeJ mice were sensitized with β-gal, alum, and pertussis toxin and subsequently received an intravenous (i.v.) β-gal challenge. Sensitizing doses and dosing intervals were optimized to induce death in 16 of 16 consecutive mice after i.v. challenge. Next, the protective effect of pACB-LacZ vaccination on outcomes after sensitization and challenge was evaluated. The timetable for vaccination, sensitization, and challenge is outlined in figure 1. As shown

in table 1, pACB-LacZ vaccination protected 5/15 mice from death in 4 experiments, while 0/16 pACB vaccinated mice survived challenge ($p = 0.018$).

Table 1

Vaccination	Survival	% Survival	p value
pACB-LacZ	5/15	33% ($p = 0.018$)	0.018
pACB	0/16	0%	--
None	0/16	0%	--

Despite the protection offered by pACB-LacZ vaccination, mice surviving challenge demonstrated behavioral changes, such as decreased activity, and rapid and labored respirations, consistent with mild anaphylactic reactions within the first 30 minutes. However, by the end of the first hour mice surviving the anaphylactic challenge appeared normal. To evaluate whether pACB-LacZ vaccination reduced mast cell degranulation from Th_2 sensitized and challenged mice, plasma histamine levels were measured before and 2 minutes after i.v. β-gal injection. Prior to anaphylactic challenge, plasma histamine levels were less then 10nM in all mice. As seen in figure 2, post challenge plasma histamine levels increased in all mice. However, they were significantly lower in pACB-LacZ vaccinated (7.4 ± 3 μM) versus pACB vaccinated (41 ± 13μM) mice ($p = 0.018$).

Example 2: pDNA vaccination effectively inhibits the development of a Th_2 biased immune response after allergen sensitization

As anaphylactic hypersensitivity is associated with Th_2 biased immunity, β-gal specific antibody and cytokine profiles of vaccinated/sensitized mice were further evaluated. As seen in figure 3A, antigen specific IgE levels were on average 15 fold lower in pACB-LacZ versus pACB vaccinated mice ($p < 0.0001$). In addition, pACB-LacZ vaccinated mice produced approximately 3 fold less IgG1($p = 0.002$) and 4 fold more antigen specific IgG2a then pACB vaccinated mice ($p = 0.0004$). Splenocytes from mice vaccinated with pACB-LacZ prior to sensitization also demonstrated significantly higher antigen specific IFNγ($p = 0.0002$) and lower IL-4 ($p = 0.024$) and IL-5 ($p = 0.012$) responses than control mice (figure 3B).

Example 3: Protein/ISS-ODN vaccination prevents the development of anaphylactic hypersensitivity

Protein/ISS-ODN co-immunization and plasmid vaccination induce similar immune profiles (18-22). Therefore, the efficacy of β-gal/ISS-ODN vaccination in protecting against the development of anaphylactic hypersensitivity was next evaluated. Mice were vaccinated with β-gal and ISS-ODN, and

then sensitized and i.v. challenged with the model allergen (figure 1). Control mice were immunized with ISS-ODN or β-gal alone, or β-gal plus M-ODN. As seen in table 2, in 3 experiments 5/12 β-gal/ISS-ODN vaccinated mice survived i.v. β-gal challenge versus 0/36 control mice receiving β-gal or ISS-ODN, or β-gal and M-ODN vaccinations ($p = 0.01$ for β-gal/ISS-ODN vaccination versus each control group).

Table 2

Vaccination	Survival	% Survival	*p* value
B-Gal + ISS-ODN	5/15	42%	0.01
ISS-ODN	0/12	0%	--
B-Gal	0/12	0%	--

Again, surviving mice demonstrated behavioral changes consistent with a mild anaphylactic reaction within the first 30 minutes, but all surviving mice appeared normal 1 hour after i.v. challenge. Plasma histamine levels, before and after challenge, were also measured. Plasma histamine levels were less then 10nM in all mice prior to challenge. As seen in figure 4, post challenge histamine levels increased in all mice, but they were significantly lower in β-gal/ISS-ODN vaccinated mice ($6.1 \pm 2.7\mu M$) then mice vaccinated with β-gal ($25.2 \pm 11\mu M$) or ISS-ODN ($26 \pm 6\mu M$) alone, or mice vaccinated with β-gal and M-ODN ($29 \pm 7.8\ \mu M$)($P < 0.05$).

Example 4: Protein/ ISS-ODN vaccination effectively inhibits the development of a Th_2 biased immune response after allergen sensitization

To establish whether β-gal/ISS-ODN vaccination prevented the development of a Th_2 biased immune profile after β-gal/alum/pertussis toxin sensitization, serum antibody and splenic cytokine profiles of β-gal/ISS-ODN vaccinated and control mice were evaluated. As seen in Figure 5A, β-gal/ISS-ODN vaccinated mice had greater than 9 fold lower serum IgE levels then control immunized mice ($p < 0.035$). In addition, although β-gal/ISS-ODN vaccinated mice had similar IgG1 levels to control mice, IgG2a levels were 8-fold higher ($p < 0.007$). β-gal/ISS-ODN vaccinated mice also produced significantly more IFNγ($p < 0.03$) and significantly less IL-4 ($p < 0.04$) and IL-5 ($p < 0.002$) than control mice (figure 5B).

Example 5: Protein/ISS vaccination reduces histamine release in allergen-sensitized mice

C3H/HeJ mice received i.d. vaccinations with 50 μg of pACB-LacZ or pACB on 3 occasions 10 days apart. Twenty five days after the last vaccination, mice were Th_2 sensitized with a mixture of

β-gal (100 μg), pertussis toxin (300 ng), and alum (1 mg) injected i.p. on 2 occasions 7 days apart. Mice then received an i.v. challenge with 150 μg of β-gal 3 weeks after their last i.p. sensitization. Plasma histamine levels were determined 2 minutes after challenge. *p* values for survival were determined by Fisher's exact test, and *p* values for histamine release were determined by unpaired 2 tailed Student's t test. Mice that died did so within 1 hour of challenge. Surviving mice demonstrated signs of anaphylaxis within the first half hour after challenge but appeared healthy 1 hour later. The results are shown in Table 3.

Table 3: Anaphylactic challenge outcomes of pACB-LacZ vaccinated mice

Vaccination	Survival	% Survival	Histamine
pACB-LacZ	5/15	33% ($p = 0.018$)	7.4 ± 3 μM ($p = 0.018$)
pACB	0/16	0%	41 ± 13 μM

As shown in Table 3, pACB-LacZ vaccination protected 5/15 mice from death in 4 experiments, while 0/16 mice vaccinated with a LacZ deficient plasmid (pACB) survived challenge. Consistent with the outcomes of challenged mice, post challenge plasma histamine levels were also significantly lower in pACB-LacZ versus pACB vaccinated mice (Table 3). Anaphylactic hypersensitivity is associated with Th2 biased immunity, and in humans is dependent on IgE production. Therefore, β-gal specific antibody and cytokine profiles of gene vaccinated/sensitized mice were further evaluated. IgE levels were on average 15-fold lower in pACB-LacZ versus pACB vaccinated mice. In addition, pACB-LacZ vaccinated mice produced approximately 3-fold less IgG1and 4-fold more antigen specific IgG2a than pACB vaccinated mice. Splenocytes from mice vaccinated with pACB-LacZ prior to sensitization further demonstrated a Th1 bias with significantly higher antigen specific IFNγ and lower IL-4 and IL-5 responses than control mice.

The efficacy of β-gal/ISS-ODN vaccination in protecting against the development of anaphylactic hypersensitivity was also evaluated. C3H/HeJ mice received i.d. vaccinations with ISS-ODN(10 μg) and β-gal (10 μg), ISS-ODN or β-gal alone or β-gal plus M-ODN (10 μg) on 3 occasions 10 days apart. Twenty five days after the last vaccination, mice were Th_2 sensitized with a mixture of β-gal (100 μg), pertussis toxin (300 ng), and alum (1 mg) injected i.p. on 2 occasions 7 days apart. Mice then received an i.v. challenge with 150 μg of β-gal 3 weeks after their last i.p. sensitization. Plasma histamine levels were determined 2 minutes after challenge. *p* values for survival were determined by Fisher's exact test, and *p* values for histamine release were determined by unpaired 2 tailed Student's t test. Mice that died did so within 1 hour of challenge. Surviving mice demonstrated signs of anaphylaxis within the first half hour after challenge but appeared healthy 1 hour later.

Mice were vaccinated with β-gal and ISS-ODN, and then sensitized and i.v. challenged with the model allergen, in a manner analogous to that described for the gene vaccination studies. One immunization control group received β-gal alone, the experimental equivalent of allergen extracts used in clinical practice. Other control mice were injected with ISS-ODN alone or β-gal plus a non-stimulatory mutated oligodeoxynucleotide (M-ODN) derived by guanine to cytosine base substitutions in the 2 CpG dinucleotides found within the parent ISS-ODN. The results are shown in Table 4.

Table 4: Anaphylactic challenge outcomes of β-gal/ISS-ODN vaccinated mice

Vaccination	Survival	% Survival	Histamine
β-gal + ISS-ODN	5/12	42% (p =0.01)	6.1 ± 2.7 μM ($p < 0.05$)
ISS-ODN	0/12	0%	25.2 ± 11 μM
β-gal	0/12	0%	26 ± 6 μM
β-gal + M-ODN	0/12	0%	29 ± 7.8 μM

As seen in Table 4, in 3 experiments 5/12 β-gal/ISS-ODN vaccinated mice survived i.v. β-gal challenge versus 0/36 control mice receiving β-gal or ISS-ODN, or β-gal and M-ODN. Post challenge histamine levels were also significantly lower in β-gal/ISS-ODN vaccinated versus control mice. Like pACB-LacZ vaccinated mice, those immunized with β-gal and ISS-ODN demonstrated a 90% decrease in serum IgE post sensitization when compared to controls. In addition, while β-gal/ISS-ODN vaccinated mice had similar IgG1 levels to control mice, IgG2a levels were 8-fold higher. Furthermore, splenocytes from β-gal/ISS-ODN vs. control vaccinated mice demonstrated an antigen specific Th1 cytokine bias reflected in elevated IFNγ and depressed IL-4 and IL-5 production upon culture with allergen.

Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a basis for modifying or designing other embodiments for carrying out the same purposes of the present invention.

CLAIMS

What is claimed is:

1. A method for reducing a symptom associated with anaphylactic hypersensitivity in a subject comprising administering to an individual a nucleic acid molecule comprising an immunomodulatory nucleic acid molecule in an amount sufficient to reduce at least one anaphylaxis-associated symptom, wherein the immunomodulatory nucleic acid molecule comprises the sequence 5'-C-G-3'.

2. The method of claim 1, wherein the immunomodulatory nucleic acid molecule is co-administered with an allergen or allergen extract.

3. The method of claim 2, wherein the immunomodulatory nucleic acid molecule and the allergen are admixed.

4. The method of claim 2, wherein the immunomodulatory nucleic acid molecule and the allergen are in proximate association.

5. The method of claim 4, wherein the immunomodulatory nucleic acid molecule is conjugated to the allergen.

6. The method of claim 1, wherein the reducing comprises reducing risk of death.

7. The method of claim 1, wherein the immunomodulatory nucleic acid molecule comprises the sequence 5'-purine-purine-C-G-pyrimidine-pyrimidine.

8. The method of claim 1, wherein the immunomodulatory nucleic acid molecule comprises the sequence 5'-purine-T-C-G-pyrimidine-pyrimidine-3'.

9. The method of claim 1, wherein the immunomodulatory nucleic acid molecule comprises the sequence 5'-(TCG)n-3', wherein n is any integer that is 1 or greater.

10. The method of claim 1, wherein the immunomodulatory nucleic acid molecule is administered via a topical, mucosal, enteral, or systemic route.

11. The method of claim 10, wherein the topical route is intranasal, ophthalmic, oral, intratracheal, intravaginal, transdermal or intrarectal.

12. The method of claim 10, wherein the systemic route is intradermal, intramuscular, or subcutaneous.

13. The method according to claim 1, wherein the allergen is a drug allergen.

14. The method according to claim 13, wherein the allergen is a food allergen.

15. The method according to claim 13, wherein the allergen is a plant allergen.

16. The method according to claim 13, wherein the allergen is an animal allergen.

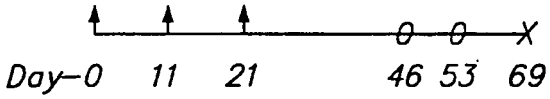
17. The method of claim 1, wherein the anaphylaxis-associated symptom is selected from the group consisting of elevated IgE level, elevated histamine level, constriction of the airways, and difficult breathing.

18. A method for reducing the risk of anaphylactic hypersensitivity in a subject comprising administering to an individual a nucleic acid molecule comprising an immunomodulatory nucleic acid molecule in an amount sufficient to reduce to reduce the risk of anaphylactic hypersensitivity, wherein the immunomodulatory nucleic acid molecule comprises a sequence selected from the group consisting of 5'-purine-purine-C-G-pyrimidine-pyrimidine, 5'-purine-T-C-G-pyrimidine-pyrimidine-3', and 5'-(TCG)n-3', wherein n is any integer that is 1 or greater.

19. A method for reducing the risk of anaphylactic shock in a subject comprising administering to an individual a nucleic acid molecule comprising an immunomodulatory nucleic acid molecule in an amount sufficient to reduce the risk of anaphylactic shock, wherein the immunomodulatory nucleic acid molecule comprises a sequence selected from the group consisting of 5'-purine-purine-C-G-pyrimidine-pyrimidine, 5'-purine-T-C-G-pyrimidine-pyrimidine-3', and 5'-(TCG)n-3', wherein n is any integer that is 1 or greater.

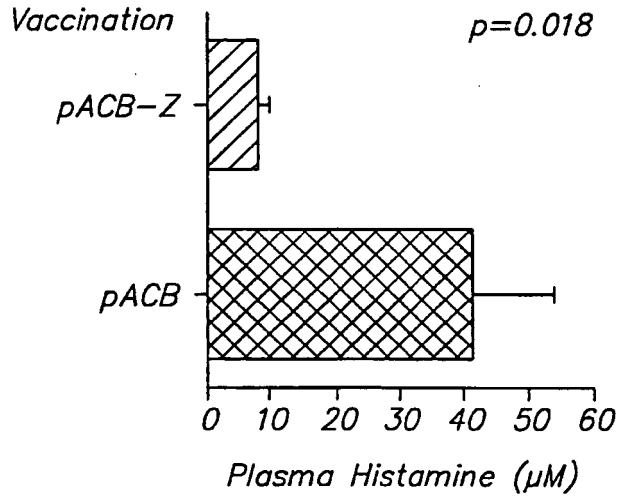
20. A method for reducing a symptom associated with anaphylactic hypersensitivity in a subject comprising administering to an individual a nucleic acid molecule comprising an immunomodulatory nucleic acid molecule in an amount sufficient to reduce at least one anaphylaxis-associated symptom, wherein the immunomodulatory nucleic acid molecule comprises a sequence selected from the group consisting of 5'-purine-purine-C-G-pyrimidine-pyrimidine, 5'-purine-T-C-G-pyrimidine-pyrimidine-3', and 5'-(TCG)n-3', wherein n is any integer that is 1 or greater.

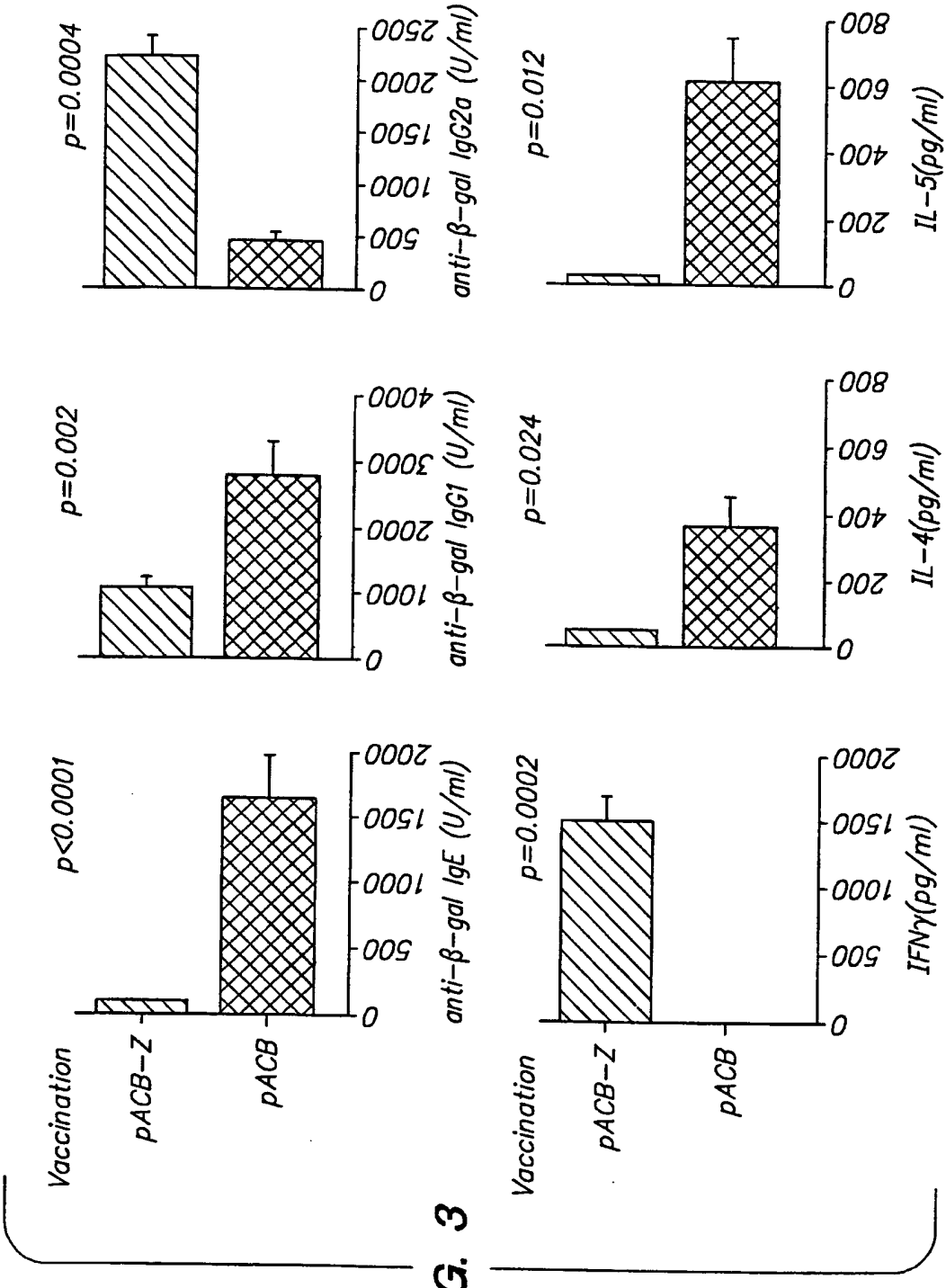
FIG. 1



↑ =DNA Based or Control Vaccination
0 =Th_2 Sensitization with β–gel, Alum, and Pertussis
X =i.v. Challenge with β–gel

FIG. 2

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1000
1500
2000
p=0.002
anti-β-gal IgG1 (U/ml)
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1000
2000
3000
4000
p=0.0004
anti-β-gal IgG2a (U/ml)
0
500
1000
1500
2000
2500
p=0.0002
IFNγ(pg/ml)
0
500
1000
1500
2000
p=0.024
IL-4(pg/ml)
0
200
400
600
800
p=0.012
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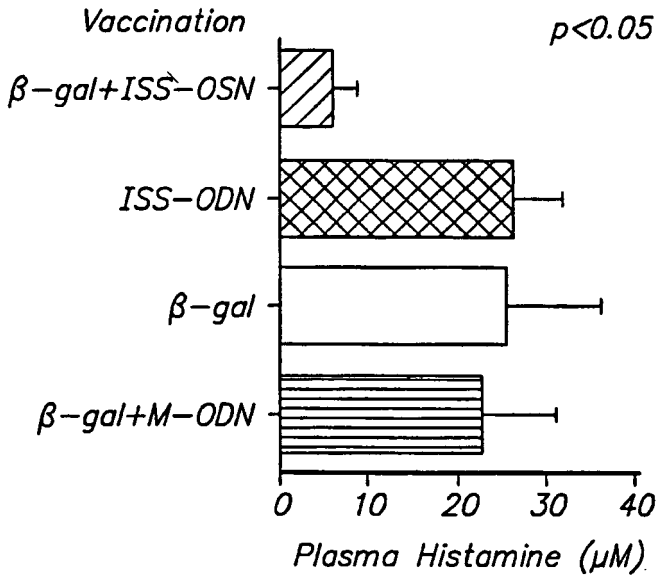
400
600
800
Vaccination
pACB-Z
pACB

FIG. 3

FIG. 4


Vaccination
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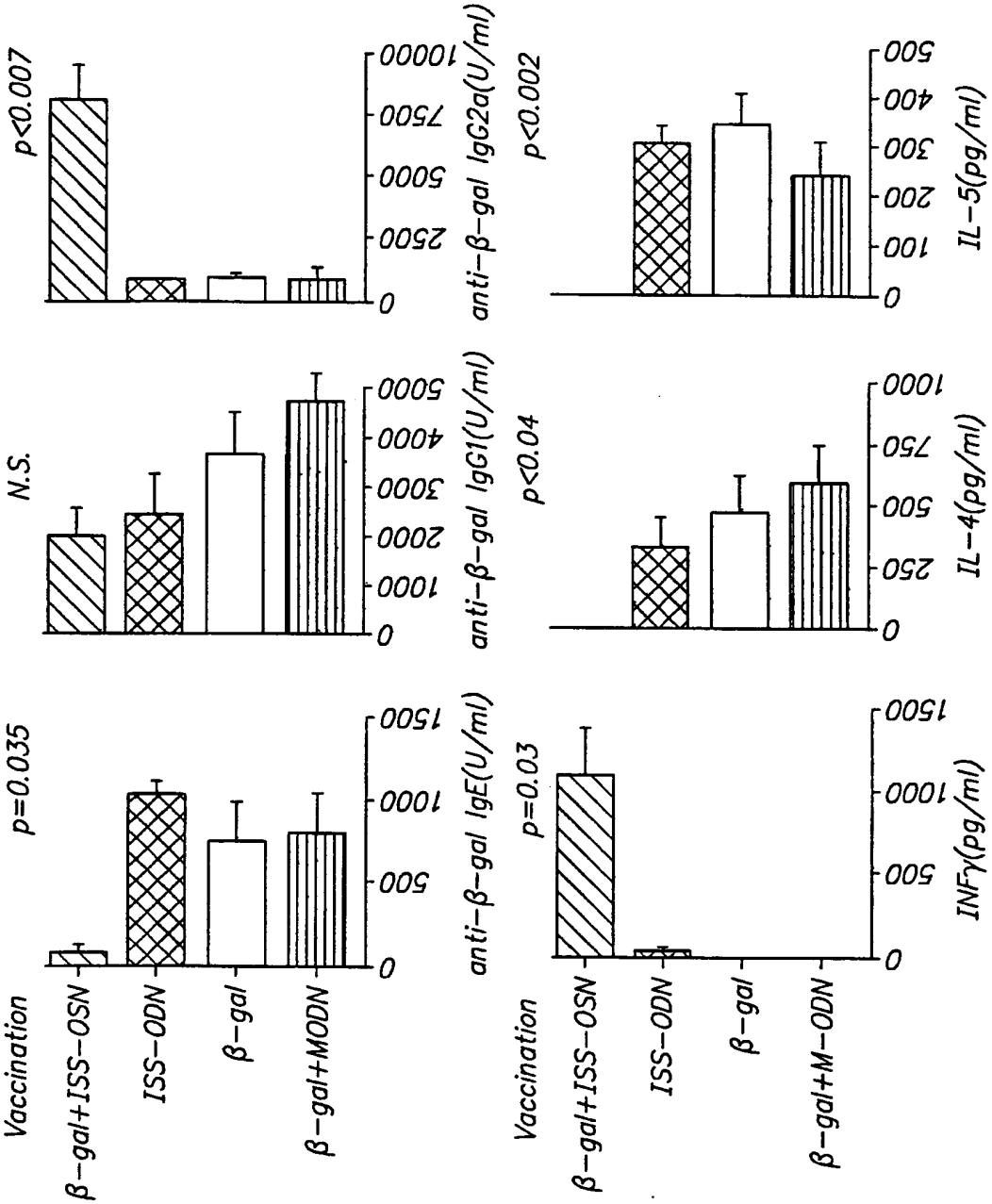
ISS−ODN
β−gal
β−gal+M−ODN
0
10
20
30
40
Plasma Histamine (μM)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5000
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0
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400
300
200
100
0
N.S.
anti-β-gal IgG1(U/ml)
5000
4000
3000
2000
1000
0
p<0.04
IL-4(pg/ml)
1000
750
500
250
0
p=0.035
anti-β-gal IgE(U/ml)
1500
1000
500
0
p=0.03
INFγ(pg/ml)
1500
1000
500
0
Vaccination
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ISS-ODN
β-gal
β-gal+MODN
Vaccination
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ISS-ODN
β-gal
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FIG. 5

SEQUENCE LISTING

<110> Raz, Eyal
Horner, Anthony A.

<120> Method for Preventing an Anaphylactic Reaction

<130> 06510/201WO1

<150> 60/171,830
<151> 1999-12-21

<160> 2

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<220>
<223> mutant

<400> 2
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/35064

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 48/00
US CL : 514/44,

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : CpG oligonucleotides, hypersensitivity

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
USPT, JPAB, EPAB, DWPI, Medline

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	SEREBRISKY et al., CpG Oligonucleotides can reverse Th2-associated allergic airway	1-7, 10, 11, 16-20
---	responses. J. Immunol. November 2000, vol. 165, No. 10, pages 5906-5912. Sections of	---
Y,P	Materials and Methods and Results.	8, 9, 12-15
X,P	SHIROTA, Regulation of murine airway esoinophilia and Th2 cells by antigen-conjugated	1-7, 10, 11, 16-20
---	CpG oligodeoxynucleotides as a nowvel antigen-specific immunomodulator. J. Immunol.	---
Y,P	June 2000, Vol 164, No. 11, pages 5575-5582. Sections of Materials and Methods and Results.	8, 9, 12-15
Y,E	US 6,194,388 A (KRIEG et al) 27 Feburary 2001(27,6, 2001), claims 13, 21.	1-4, 7-9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
12 March 2001 (12.03.2001)

Date of mailing of the international search report
20 APR 2001

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Form PCT/ISA/210 (second sheet) (July 1998)